REMARKS

Supplemental Material:

Applicants have provided additional material with this Amendment and Response in the form of a 37 CFR 1.132 Declaration. The material consists of experiments performed using Applicants' processes providing delivery of polynucleotides to cells *in vivo* to inhibit gene expression. The experiments show: (a) inhibition of endogenous genes; cytosolic alanine aminotransferase, peroxisome proliferators-activated receptor, HMG Coenzyme A reductase, and green fluorescent protein; (b) delivery of polynucleotide to heart, skeletal muscle, prostate and lung; (c) images demonstrating delivery of fluorescently labeled siRNA to liver; and (d) evidence that inhibition of gene expression by the delivered polynucleotide is not dependent on interaction of the polynucleotide with a co-delivered plasmid prior to delivery. The experiments used the processes described in the instant application and introduce no new matter.

Rejection of claims under 35 USC § 112:

Claim 8 has been rejected as being indefinite because of insufficient antecedent basis for the limitation "the parenchymal cell." Applicants have amended claim 8 to recite " a cell" to obviate the rejection.

Claims 1-16 have been rejected under USC 112 first paragraph for lack of enablement commensurate with the scope of the claims.

On page 3, first paragraph, the Action rejects Claims 1-16 because the Specification does not enable any person skilled in the art to use the invention within the scope of the claims. With this Amendment and Response, Applicants provide further evidence in the form of Supplemental Information showing enablement. Applicants request reconsideration of the rejection based upon the new information.

The office action further states on page 3 that methodologies are provided for inhibiting gene expression in the liver, spleen, lung and kidney. Applicants respectfully point out that examples 6 and 9 demonstrate delivery of polynucleotides to heart cells and skeletal muscle cells, respectively, to inhibit gene expression.

The office action also states on page 3 that the methodologies in the specification only disclose delivery of siRNA together with a plasmid containing the sequence to be inhibited. Applicants respectfully point out that delivery of siRNA without co-delivery of a plasmid is demonstrated in example 11.

Applicants utilized co-delivery with a plasmid containing an expressible reporter gene as a convenient method to quantitatively assay delivery of the siRNA. The invention does not require co-delivery of a plasmid for delivery of siRNA and absence of plasmid DNA in the injection solution will not effect siRNA delivery. As evidence for this assertion, the results in example 11 show inhibition of an endogenous gene following delivery of siRNA without co-delivery of plasmid DNA.

As further evidence of enablement, Applicants submit Supplemental Information showing delivery of polynucleotide to inhibit gene expression to various tissues and inhibition of both co-delivered and endogenous genes. The Supplemental Information shows (a) inhibition of endogenous genes; cytosolic alanine aminotransferase, peroxisome proliferators-activated receptor, HMG Coenzyme A reductase, and green fluorescent protein; (b) delivery of siRNA to heart, skeletal muscle, prostate and lung; (c) images demonstrating delivery of fluorescently labeled siRNA to liver; and (d) evidence that inhibition of gene expression by the delivered siRNA is not dependent on interaction of the siRNA with a co-delivered plasmid prior to delivery. The experiments used the processes described in the instant application and introduce no new matter.

Applicants agree that previously described methods for delivering antisense molecules, ribozymes, etc. to cells *in vivo*, have failed to reproducibly deliver these molecules to cells to affect gene expression. However, Applicants have developed a delivery system which overcomes previous limitations.

The office action states on page 3, citing a reference by Verma (1997), that vector targeting to desired tissues *in vivo* continues to be unpredictable and inefficient. Verma mentions, in one paragraph, that direct injection of DNA into a tissue, polylysine/DNA complexes and cationic lipid/DNA complexes suffer from poor delivery efficiency. The remainder of the article covers problems associated with various viral vectors. It is important to note that none of the

methods discussed by Verma encompass the method taught by the Applicants in the instant application.

As the Office Action notes, Crystal (1995) states that increasing the efficiency of gene transfer is a hurdle. Crystal also concludes by stating that "none of the drug development problems facing human gene transfer are insurmountable... ." As with Verma, Crystal discusses the problems associated with viral vectors and plasmid/liposome complexes, but does not mention any delivery process encompassed by the Applicants' invention.

The Office Actions points to statement by both Bass (2001) and Tuma (2003) that delivery of siRNA to cells *in vivo* will run into the same problems as delivery of antisense molecules. Bass compares delivery in mammals with delivery to *C. elegans*. "Double strand RNA can be delivered to *C. elegans* by feeding or soaking." By comparison, nearly any delivery method could be considered difficult. Tuma states that getting the nucleic acids into target cells will be a problem. However, as Tuma does not address any particular delivery system or vector, it is impossible to know if Tuma has contemplated a method for delivering siRNA or antisense RNA to cells as taught by the Applicants.

The Action cites Branch (1998) which indicates that there is a high level of unpredictability with antisense molecules. In particular, Branch discusses at length, the problems associated with identifying a good antisense nucleic acid for specifically inhibiting a given gene. However, Applicants have not claimed a process for predicting the qualifying characteristics of an antisense molecule. Applicants disclose that using their invention, one practicing the art would be able to deliver a given antisense molecule to a cell *in vivo* to (a) inhibit gene expression if the antisense molecule is known to be effective; or (b) determine if the antisense molecule is effective in inhibiting gene expression *in vivo* if its efficacy in unknown.

Additionally, identifying effective siRNA's is more straightforward than identifying effective antisense sequences. Aza-Blanc et al. 2003 Molecular Cell 12:627-637 reported greater than 70% inhibition with over 60% of the siRNA's they tested (See section titled *Evaluation of siRNA Activity* on page 627-628).

The Office Action states on page 5-6 that it is well known in the art that oligonucleotides delivered systemically are known to concentrate in the liver (Ma et al 2000). Ma et al. are

referring to the process of clearance of foreign substances circulating in the blood. Clearance of molecules from the blood stream in this manner is accomplished by a pathway that specifically involves the degradation and/or excretion of the molecule from the body. In contrast, Applicants provide a process for delivering siRNA to a cell of an organ such that the siRNA is available to inhibit expression of a gene in the cell. Our studies and others indicate that simply perfusing siRNA into the bloodstream is insufficient to deliver the siRNA to cells. Applicants have found that perfusion of labeled siRNA into the bloodstream of mice fails to efficiently deliver the siRNA to the liver. Injecting the siRNA into the tail vein and increasing permeability of the vessel, however, results in delivery of the labeled siRNA to hepatocytes (see Supplemental Information). Boutla et al. 2001 (Current Biology 11:1776-1780, IDS reference) similarly indicate that simply inserting siRNA into mouse fails to deliver functional siRNA to cells in the mammal (see the final paragraph on page 1779).

The office action states that "it is very likely that the highly concentrated siRNA will inhibit its target gene well before it is systemically delivered." Applicants respectfully disagree. SiRNA has been shown to effect inhibition of gene expression by degrading RNA in the cytoplasm of the cell. Since DNA must first be transcribed to produce an RNA which is then processed to a mature mRNA before siRNA can function, siRNA would not have any effect prior to delivery. We have shown, as indicated in the Supplemental Information, that control siRNA with sequence identical to another section of the co-delivered plasmid (siRNA-ori) has no effect on expression of the marker gene. Furthermore, control siRNA with sequence complementary to an intron (within the same transcriptional unit and present in the primary transcript but not the mature mRNA) of a co-delivered marker gene, also had no inhibitory effect on expression of the gene. These two observations demonstrate that interaction of the siRNA with the plasmid prior to injection of the two nucleic acids into the animal is not responsible for inhibition of expression of the plasmid encoded gene.

<u>Double Patenting Rejection</u>

Claims 1-5, 8, 13 and 15 have been rejected as being unpatentable over US Patent No. 6,349,966. Applicants have amended this application to become a CIP from their earlier application. Specifically this application has been amended to claim direct benefit of application 09/391,260 filed on Sep. 7, 1999, which is a Continuation of 08/975,573 filed Nov. 21, 1997, now Pat. No. 6,265,387, which is a continuation of 08/571,536 filed on December 13, 1995, now abandoned. The current application becomes a Continuation-In-Part

of the original 1995 filing based upon use of its vascular delivery processes. A copy of the '536 application is enclosed for review. In view of the amendment, the double patenting rejection is believed to be obviated.

Rejection of claims under 35 USC § 102:

Claims 1-6, 8, 13 and 15 have been rejected under 35 U.S.C. 102(b) as being anticipated by Makino et al. 1998. Claims 1-5, 9, 10, 13, 14 and 15 have been rejected under 35 U.S.C. 102(b) as being anticipated by Wianny et al 2000. Applicants have amended this application to become a CIP from their earlier application. Specifically this application has been amended to claim direct benefit of application 09/391,260 filed on Sep. 7, 1999, which is a Continuation of 08/975,573 filed Nov. 21, 1997, now Pat. No. 6,265,387, which is a continuation of 08/571,536 filed on December 13, 1995, now abandoned. The current application becomes a Continuation-In-Part of the original 1995 filing based upon use of its vascular delivery processes. A copy of the '536 application is enclosed for review. In view of the amendment, the rejection is believed to be obviated.

The Examiner's objections and rejections are now believed to be overcome by this response to the Office Action. In view of Applicants' amendment and arguments, it is submitted that claims 1 and 3-16 should be allowable.

Respectfully submitted,

Mark K. Johnson Reg. No. 35,909
Mirus
505 South Rosa Road
Madison, WI 53719
608-238-4400

I hereby certify that this correspondence is being sent by United States Postal Service Express Mail to: Commissioner for Patents, PO Box 1450, Alexandria, VA 22313-1450 on this date: Dec. 16, 2003.

Kirk Ekena

Inhibition Of Gene Expression By Delivery Of Small Interfering RNA To Post-Embryonic Animal Cells *In vivo*

This Patent Application is related to ~~pending United States~~ provisional patent applications 60/315,394 filed August 27, 2001~~,~~ and 60/324,155 filed September 20, 2001; and is a Continuation-In-Part of United States Patent Applications serial numbers 09/707,117 filed November 6, 2000 and 09/391,260 filed on Sep. 7, 1999, which is a Continuation of 08/975,573 filed Nov. 21, 1997, now Pat. No. 6,265,387, which is a continuation of 08/571,536 filed on December 13, 1995, now abandoned.

FIELD

The present invention generally relates to inhibiting gene expression. Specifically, it relates to inhibiting gene expression by delivery of small interfering RNAs (siRNAs) to post-embryonic animals.

BACKGROUND

RNA interference (RNAi) describes the phenomenon whereby the presence of double-stranded RNA (dsRNA) of sequence that is identical or highly similar to a target gene results in the degradation of messenger RNA (mRNA) transcribed from that targeted gene (Sharp 2001). RNAi is likely mediated by siRNAs of approximately 21-25 nucleotides in length which are generated from the input dsRNAs (Hammond, Bernstein et al. 2000; Parrish, Fleenor et al. 2000; Yang, Lu et al. 2000; Zamore, Tuschl et al. 2000; Bernstein, Caudy et al. 2001).

The ability to specifically knock-down expression of a target gene by RNAi has obvious benefits. For example, RNAi could be used to generate animals that mimic true genetic "knockout" animals to study gene function. In addition, many diseases arise from the abnormal expression of a particular gene or group of genes. RNAi could be used to inhibit the expression of the genes and therefore alleviate symptoms of or cure the disease. For example, genes contributing to a cancerous state could be inhibited. In addition, viral genes could be inhibited, as well as mutant genes causing dominant genetic diseases such as myotonic dystrophy. Inhibiting such genes as cyclooxygenase or cytokines could also treat inflammatory diseases such as arthritis. Nervous system disorders could also be treated. Examples of targeted organs would include the liver, pancreas, spleen, skin, brain, prostrate, heart etc.